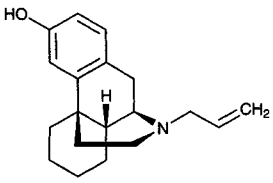
Levallorphan



Molecular formula: $C_{19}H_{25}NO$
Molecular weight: 283.41
CAS Registry No.: 152-02-3, 71-82-9 (tartrate)
Merck Index: 5485

SAMPLE

Matrix: blood, CSF, urine
Sample preparation: Dilute urine 3:1 or more with water. Mix 1 mL CSF, plasma, or diluted urine with 500 μL saturated sodium carbonate, add 5 mL hexane containing 0.1% n-octylamine. Vortex for 60 s, centrifuge at 2000 g for 10 min. Re-extract aqueous phase with 5 mL hexane containing 0.1% n-octylamine, evaporate the combined hexane extracts to dryness under a stream of nitrogen in a 50° water bath. Reconstitute residue with 150 μL 100 mM hydrochloric acid, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm CN
Column: 220 × 4.6 5 μm Brownlee Spheri-5CN (Applied Biosystems, USA)
Mobile phase: MeCN:n-octylamine:water 19:0.05:80.95 adjusted to pH 2.8 with phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: F ex 230 em 330

CHROMATOGRAM

Retention time: 7.4
Internal standard: levallorphan

OTHER SUBSTANCES

Extracted: dextromethorphan

KEY WORDS

plasma; levallorphan is IS

REFERENCE

Kimiskidis,V.K.; Kazis,A.D.; Niopas,I. Simultaneous determination of dextromethorphan and dextrorphan in human plasma, urine and cerebrospinal fluid by HPLC with fluorescence detection, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 1267–1275.

SAMPLE

Matrix: blood, saliva, urine
Sample preparation: Add 100 μL 28% ammonium hydroxide and 5 mL n-butanol:hexane 10:90 to 5 mL urine, 1 mL plasma or 3 mL saliva. Rotate for 30 min and centrifuge at 4500 rpm for 10 min. Remove the upper organic layer and extract it with 300 μL 100 mM HCl by vortexing for 20 min, centrifuge for 5 min. Inject a 40 μL (urine) or 200 μL (plasma, saliva) aliquot of the acidic layer.

HPLC VARIABLES

Column: Zorbax RP-phenyl
Mobile phase: MeCN:10 mM potassium phosphate 50:50 adjusted to pH 4.0 with 8.5% phosphoric acid
Column temperature: 40
Flow rate: 1.0
Injection volume: 40-200
Detector: F ex 280 em 310

CHROMATOGRAM

Retention time: 8.6

Internal standard: levallorphan

OTHER SUBSTANCES
Extracted: dextromethorphan

KEY WORDS
levallorphan is IS; plasma

REFERENCE
Hu,O.Y.-P.; Tang,H.-S.; Lane,H.-Y.; Chang,W.-H.; Hu,T.-M. Novel single-point plasma or saliva dextromethorphan method for determining CYP2D6 activity, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 955–960.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL MeOH:water:zinc sulfate 50:45:5, centrifuge at 10000 g for 3 min, extract the supernatant twice with 3 mL dichloromethane. Combine dichloromethane extracts, dry under a stream of nitrogen, dissolve the residue in 200 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm polymer C18 (Astec, Whippany, NJ)
Mobile phase: MeCN:50 nM (sic) pH 9.0 ammonium carbonate buffer 60:40 (Buffer was adjusted to pH 9.0 with ammonium hydroxide.)
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV (wavelength not given)

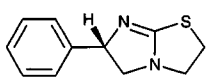
CHROMATOGRAM
Retention time: 11.35
Internal standard: levallorphan

OTHER SUBSTANCES
Extracted: dextromethorphan, dextrorphan

KEY WORDS
liver; levallorphan is IS

REFERENCE
Vielnascher,E.; Spatzenegger,M.; Mayrhofer,A.; Klinger,P.; Jäger,W. Metabolism of dextromethorphan in human liver microsomes: a rapid HPLC assay to monitor cytochrome P450 2D6 activity, *Pharmazie*, **1996**, *51*, 586–588.

Levamisole



Molecular formula: $C_{11}H_{12}N_2S$
Molecular weight: 204.30
CAS Registry No.: 14769-73-4, 16595-80-5 (HCl), 5036-02-2 (racemic), 5086-74-8 (racemic HCl)
Merck Index: 5486
Lednicer No.: 4 217

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 6.97

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell cultures
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 20 mL water. Add 2 mL cell culture to the SPE cartridge, wash with 20 mL water, elute with 10 mL MeOH:water 80:20, inject a 10 μL portion of the eluate.

HPLC VARIABLES
Column: 100 × 8 4 μm RCM Nova-Pak C18
Mobile phase: MeCN:100 mM ammonium acetate 46:54
Flow rate: 1.3
Injection volume: 10
Detector: UV 245

CHROMATOGRAM
Retention time: 2.2
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Shu,Y.-Z.; Kingston,D.G.I.; Van Tassell,R.L.; Wilkins,T.D. Metabolism of levamisole, an anti-colon cancer drug, by human intestinal bacteria, *Xenobiotica*, **1991**, *21*, 737–750.

SAMPLE
Matrix: milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate, with 10 mL MeOH, and with 10 mL water. Acidify 50 mL milk to pH 4.6 with 6 M HCl, centrifuge at 10° at 4200 g for 15 min, remove the supernatant from the solid and the fat layer. Adjust

the pH of the supernatant to 11.0-11.2 with a few drops of 40% NaOH, add a 25 mL aliquot to the SPE cartridge, elute with 10 mL water-saturated ethyl acetate. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute the residue in 200 μL mobile phase.

HPLC VARIABLES
Guard column: normal phase universal guard cartridge kit (Whatman)
Column: 250 × 2.1 Spherisorb S5W
Mobile phase: Dichloromethane:MeOH 95:5
Flow rate: 0.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12

KEY WORDS
normal phase; SPE; detection with GC for higher sensitivity

REFERENCE
Chappell,C.G.; Creaser,C.S.; Shepherd,M.J. Modified on-column interface for coupled high-performance liquid chromatography-gas chromatography and its application to the determination of levamisole in milk, *J.Chromatogr.*, **1992**, *626*, 223–230.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone,

naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject 10 μL of a solution in RPMI-1640.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil MS C8
Mobile phase: MeCN:water 75:25 containing 0.02% dimethyloctylamine and 0.02% trifluoroacetic acid
Injection volume: 10
Detector: radioactivity

CHROMATOGRAM

Retention time: 9.9

KEY WORDS

tritium labelled

REFERENCE

Ho,N.F.H.; Sims,S.M.; Vidmar,T.J.; Day,J.S.; Barsuhn,C.L.; Thomas,E.M.; Geary,T.G.; Thompson,D.P. Theoretical perspectives on anthelmintic drug discovery: Interplay of transport kinetics, physicochemical properties, and in vitro activity of anthelmintic drugs, *J.Pharm.Sci.*, **1994**, *83*, 1052–1059.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL Bakerbond Cyano SPE cartridge with 5 mL ethyl acetate:hexane 50:50, do not allow to dry out. Pulverize frozen tissue samples to a fine powder. 3 g Powdered tissue + 2 g anhydrous sodium sulfate + 9 mL ethyl acetate + 500 μL 50% KOH (w/v), homogenize (Silverson) for 1 min, centrifuge at 2000 rpm for 10 min. Remove 6 mL of the ethyl acetate extract and add it to 6 mL hexane, mix, add to the SPE cartridge, wash with 5 mL chloroform:hexane 50:50, dry under vacuum for 10 min, elute with two 2 mL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 200 μL, sonicate for 5 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm LiChrospher 60 RP-select B guard column
Column: 125 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeCN:THF:triethylamine:water 35:5:0.2:59.8 containing 100 mM ammonium acetate
Flow rate: 1
Injection volume: 50

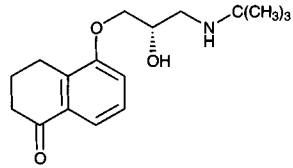
Detector: MS, Hewlett-Packard 5989A, thermospray, vaporizer 15° below take-off temperature, ion source 250°, quadrupole 100°, electron multiplier +200 V with respect to autotune voltage, SIM, m/z 205

CHROMATOGRAM
Retention time: 3.5
Limit of detection: <3 ng/g

KEY WORDS
sheep; liver; kidney; muscle; SPE; LC-MS; thermospray

REFERENCE
Cannavan,A.; Blanchflower,W.J.; Kennedy,D.G. Determination of levamisole in animal tissues using liquid chromatography-thermospray mass spectrometry, *Analyst*, **1995**, *120*, 331–333.

Levobunolol



Molecular formula: $C_{17}H_{25}NO_3$
Molecular weight: 291.39
CAS Registry No.: 47141-42-4, 27912-14-7 (HCl)
Merck Index: 5488
Lednicer No.: 2 110, 215, 280

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. Add MeCN to blood so that the ratio is 1:5. Remove a 1 mL aliquot and add 10 μL 2 μg/mL metoprolol in water, add 1 mL water, adjust pH to 9.8-10.2 with 7-10 drops 100 mM NaOH, add 2 mL benzene (CAUTION! Benzene is a carcinogen!), shake on an automatic shaker for 30 min, centrifuge, remove the organic phase and extract the aqueous layer again with 2 mL benzene for 20 min. Combine the organic layers and evaporate them under reduced pressure at 30°, dissolve the residue in 50 μL mobile phase, inject a 10-50 μL aliquot and determine amount of dihydrolevobunolol present (DHL-A). Add MeCN to blood so that the ratio was 1:5. Remove a 1 mL aliquot and add 10 μL 2 μg/mL metoprolol in water, add 1 mL water, adjust pH to 9.8-10.2 with 7-10 drops 100 mM NaOH, add 2 mL benzene, shake on an automatic shaker for 30 min, centrifuge, remove the organic phase and extract the aqueous layer again with 2 mL benzene for 20 min. Combine the organic layers and evaporate them under reduced pressure at 30°, dissolve the residue in 200 μL MeOH, add 5 mg sodium borohydride, let stand at room temperature in a closed tube for 30 min, add 1 mL water, add 300 mg NaCl, extract with 3 mL benzene for 20 min, centrifuge. Remove the organic layer and evaporate it under reduced pressure, dissolve the residue in 50 μL mobile phase, inject a 10-50 μL aliquot and determine the amount of dihydrolevobunolol (DHL-B) that is now present which represents the total amount of levobunolol and dihydrolevobunolol originally present. Determine amount of levobunolol originally present by subtracting DHL-A from DHL-B. Urine. 20-1000 μL Urine + 200 ng metoprolol + 1 mL 200 mM pH 10.2 sodium borate buffer (Sörensen), adjust pH to 9.8-10.2 with 100 mM NaOH (if necessary), add 500 mg NaCl, extract with 4 mL benzene for 30 min, proceed as described above for blood samples. (Levobunolol itself is not fluorescent so it must be reduced to the fluorescent derivative dihydrolevobunolol. Addition of MeCN to freshly drawn blood stopped enzymatic conversion of levobunolol to dihydrolevobunolol.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:water 48:52 containing 0.4% phosphoric acid and 0.2% heptanesulfonic acid
Flow rate: 2
Injection volume: 10-50
Detector: F ex 225 em 295

CHROMATOGRAM
Retention time: 3.8 (dihydrolevobunolol)
Internal standard: metoprolol (4.6)
Limit of detection: 0.5-1 ng/mL

KEY WORDS
human; dog

REFERENCE
Hengy,H.; Kölle,E.-U. Determination of levobunolol and dihydrolevobunolol in blood and urine by high-performance liquid chromatography using fluorescence detection, *J.Chromatogr.*, **1985**, *338*, 444–449.

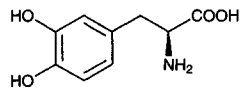
SAMPLE
Matrix: solutions
Sample preparation: Dilute in an appropriate solvent, inject an aliquot.

HPLC VARIABLES
Guard column: RC18 Guardpak (Waters)
Column: 150 × 4.5 5 μm Altex C18
Mobile phase: MeOH:water:phosphoric acid 48:52:0.1, containing 2 g/L sodium heptanesulfonate, pH adjusted to 3.5 with 3 M NaOH
Flow rate: 1.5
Detector: UV 255

CHROMATOGRAM
Retention time: 5-5.3

REFERENCE
Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

Levodopa



Molecular formula: $C_9H_{11}NO_4$
Molecular weight: 197.19
CAS Registry No.: 59-92-7
Merck Index: 5490

SAMPLE
Matrix: amniotic fluid, blood, CSF, urine
Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the

ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 34.14
Internal standard: norleucine (55.07)

OTHER SUBSTANCES

Extracted: β-alanine, alanine, alloisoleucine, α-aminoadipic acid, 4-aminobenzoic acid, gamma-aminobutyric acid, β-amino-n-butyric acid, gamma-amino-n-butyric acid, 4-aminohippuric acid, β-aminoisobutyric acid, 4-aminophenylacetic acid, α-aminophenylacetic acid, 3-amino-3-phenylpropionic acid, δ-amino-n-valeric acid, ammonia, anserine, arginine, asparagine, aspartic acid, aspartylglucosamine, carnosine, citrulline, cystathionine, cysteic acid, cysteine, cysteine-homocysteine (mixed disulfide), cystine, ethanolamine, ethionine, ethylamine, galactosamine, glucosamine, glutamic acid, glutamine, glutathionine (oxidized), glycine, glycylglycine, glycylhistidine, glycylleucine, glycylphenylalanine, glycyltyrosine, histidine, homoarginine, homocitrulline, homoserine, homocystine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine, hydroxyproline, isoleucine, kynurenine, leucine, lysine, methionine sulfone, methionine, 3-methylhistidine, 1-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, serotonin, taurine, threonine, tromethamine, tryptophan, tyrosine, valine
Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS

derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE

Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE

Matrix: blood
Sample preparation: Mix 450 μL plasma with 50 μL 310 μ g/L iso-homovanilic acid in antioxidant solution. Dialyze this solution using a Carnegie Medicin (Stockholm) microdialysis system. Perfuse 10 mm microdialysis probes with Ringer solution at 2 μ L/min. Collect dialysate for each plasma sample over 20 min in a vial containing 80 μL antioxidant solution, inject a 100 μL aliquot. (Antioxidant solution was 10 mM HCl containing 1 g/L sodium metabisulfite and 0.1 g/L Na_2EDTA.)

HPLC VARIABLES

Guard column: 30 × 4 Bondapak C18/Corasil 37-50 μ m
Column: 250 × 4.8 5 μm ODS (Beckman, CA)

Mobile phase: MeOH:buffer 20:80 (Buffer was 70 mM pH 2.55 NaH_2PO_4 containing 2.08 mM sodium octanesulfonate and 80 μ M EDTA.)
Flow rate: 1
Injection volume: 100
Detector: E , Waters 460 containing an electrochemical cell fitted with a glassy carbon working electrode and an Ag/AgCl reference electrode; the detector potential is + 0.8 V vs the reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: iso-homovanilic acid (10)
Limit of detection: 1.1 nM/L

OTHER SUBSTANCES
Extracted: dihydroxyphenylacetic acid, dopamine, homovanilic acid

KEY WORDS
plasma; pharmacokinetics; dialysate

REFERENCE
Dethy,S.; Laute,M.A.; Van Blercom,N.; Damhaut,P.; Goldman,S.; Hildebrand,J. Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients, *Clin.Chem.*, **1997**, *43*, 740–744.

SAMPLE
Matrix: blood
Sample preparation: Total levodopa. Add 300 μL 60 mM trichloroacetic acid to 1 mL plasma, place the mixture in an ice bath for 10 min, centrifuge at 5000 g for 10 min, inject a 20 μL aliquot of the supernatant. Unbound levodopa. Add 250 μL plasma to 5 μL 4 M orthophosphoric acid, centrifuge through a membrane Minicent 10 (Bio-Rad) at 8000 g at 25° for 30 min, inject a 20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 45 × 4.6 5 μm C18 (Bio-Rad)
Mobile phase: MeCN:1.4 mM sodium dodecylsulphate:50 mM KH_2PO_4 16:42:42, pH 2.8
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem 5100, analytical cell +50 mV, -300 mV, conditioning cell +300 mV

CHROMATOGRAM
Retention time: 3.7
Limit of detection: 200 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Rizzo,V.; Memmi,M.; Moratti,R.; Melzi,E.; Perucca,E. Concentrations of L-dopa in plasma and plasma ultrafiltrates, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1043–1046.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 μL 4 M perchloric acid + 50 μL 1 μg/mL dihydroxybenzylamine in 0.1 M perchloric acid, centrifuge at 1500 g for 10 min. Remove 300 μL supernatant and centrifuge it at 1600 g through a 0.2 μm regenerated cellulose filter, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Biophase ODS + 50 × 4.6 Pelliguard LC-18

Column: 250 × 4.6 5 μm Biophase ODS or 250 × 4.6 Phase II ODS (both from Bioanalytical Systems)
Mobile phase: MeOH:buffer 5:95 (Buffer was 20 mM sodium citrate, 100 mM NaH_2PO_4, 0.15 mM, and 1.25 mM heptanesulfonic acid, pH 3.2.)
Column temperature: 28
Flow rate: 1-1.5
Injection volume: 20
Detector: E, Bioanalytical Systems LC-150 in dual-parallel mode, channel 1 700 mV 200 nA f.s. for levodopa and 3-O-methyldopa, channel 2 560 mV 10 nA f.s. for dopamine, carbidopa, and dihydroxyphenylacetic acid, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.1
Internal standard: dihydroxybenzylamine (7)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: 3-O-methyldopa, dopamine, carbidopa, dihydroxyphenylacetic acid

KEY WORDS
plasma

REFERENCE
Cedarbaum,J.M.; Williamson,R.; Kutt,H. Simultaneous determination of levodopa, its metabolites and carbidopa in clinical samples, *J.Chromatogr.*, **1987**, *415*, 393–399.

SAMPLE
Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex preswollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. Add 1 mL plasma to column, elute with 5.5 mL water, discard first 1 mL. Add next 4.5 mL to 0.5 mL 0.5 M perchloric acid, centrifuge, inject 10 μL aliquot of supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid
Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: O-methyldopa, carbidopa, dihydroxyphenylacetic acid (DOPAC)

KEY WORDS
plasma

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 100 μL 4 M perchloric acid, vortex, centrifuge at 2000 g for 10 min, inject a 60 μL aliquot of the supernatant (keep sample tray at 6 ± 1°).

HPLC VARIABLES

Guard column: 45 × 4.6 37-40 μm Whatman pellicular-ODS followed by 45 × 4.6 5 μm Ultra sphere-IP C18
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:20 mM orthophosphoric acid and 4 mM sodium octanesulfonate 25:75 adjusted to pH 2.8 ± 0.05 with 50% NaOH
Column temperature: 40
Flow rate: 1
Injection volume: 60
Detector: E, BAS LC-4B, 0.75 V vs Ag/AgCl, 5 nA full scale for carbidopa, 20 nA full scale for 3-O-methyldopa and levodopa

CHROMATOGRAM

Retention time: 5.9
Internal standard: α-ethyldopa (15.4)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: carbidopa, 3-O-methyldopa

KEY WORDS

plasma; stabilize plasma sample immediately with EDTA and 2 mg/mL sodium metabisulfite

REFERENCE

Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

SAMPLE

Matrix: blood
Sample preparation: 4 mL Plasma + 500 μL 20 mg/mL ascorbic acid solution, vortex for 30 s. 1 mL Aliquot + 75 mg acid washed alumina + 100 μL 1 μg/mL 3,4-dihydroxybenzylamine hydrobromide in buffer, vortex, add 1 mL 1.5 M pH 8.6 TRIS buffer, shake at 230 oscillations/min for 15 min. Allow to settle and discard plasma, wash the alumina twice by shaking with 5 mL water for 10 min. To the washed alumina add 900 μL buffer, vortex for 20 s, allow to settle, inject a 50 μL aliquot of the supernatant. (Buffer was 200 mM phosphoric acid containing 3.3 μm EDTA and 6.7 μm potassium metabisulfite.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:buffer 8:92 (Buffer was pH 2.6 550 mM NaH_2PO_4 containing 1 mM sodium octyl sulfate and 0.7 mM EDTA.)
Flow rate: 1.5
Injection volume: 50
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode, Ag/AgCl reference electrode, 0.75 V.

CHROMATOGRAM

Retention time: 3.3
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (4.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: carbidopa
Noninterfering: caffeine, ibuprofen, aspirin, nicotine, acetaminophen, theophylline

KEY WORDS

plasma; SPE

REFERENCE
Miller,R.B.; Dehelean,L.; Bélanger,L. Determination of carbidopa and levodopa in human plasma by high-performance liquid chromatography with electrochemical detection, *Chromatographia*, **1993**, *35*, 607–612.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 2 μg/mL 3,4-dihydroxybenzylamine in 0.4 M perchloric acid + 25 μL 70% perchloric acid, vortex 1 min, keep on ice for 1 min, store at -80°. Allow to thaw at +4°, vortex 1 min, centrifuge at 1200 g at +4° for 15 min. Remove 300 μL supernatant and add it to 200 μL 2 M pH 4.5 potassium citrate buffer, centrifuge at 1200 g at +4° for 10 min, inject 50 μL of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:MeCN:buffer 8:4:88 (Buffer was 50 mM pH 3.2 phosphate containing 3.5 mM heptanesulfonic acid and 0.05 mM EDTA.)
Flow rate: 1
Injection volume: 50
Detector: E, ESA Model 5100, Model 5020 guard cell +0.6 V, Model 5010 analytical cell, DET 1 +0.35 V, DET 2 -0.35 V, both DET 1 and DET 2 monitored

CHROMATOGRAM
Retention time: 4.15
Internal standard: 3,4-dihydroxybenzylamine (8.26)
Limit of detection: 19.6 ng/mL

OTHER SUBSTANCES
Simultaneous: 3-O-methyldopa, dopamine, L-DOPA methyl ester

KEY WORDS
plasma; rat; human

REFERENCE
Rondelli,I.; Acerbi,D.; Mariotti,F.; Ventura,P. Simultaneous determination of levodopa methyl ester, levodopa, 3-O-methyldopa and dopamine in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1994**, *653*, 17–23.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 20 μL Plasma + 180 μL isoproterenol in 100 mM perchloric acid containing 10 μM disodium EDTA, centrifuge at 4° at 4000 rpm for 10 min. Filter (0.45 μm cellulose acetate) the supernatant and inject a 10 μL aliquot of the supernatant. Tissue. Homogenize (Polytron PT 10-35) 50 mg brain tissue + 500 μL isoproterenol in 100 mM perchloric acid containing 10 μM disodium EDTA at 15000 rpm for 10 s, centrifuge at 4° at 4000 rpm for 10 min. Filter (0.45 μm cellulose acetate) the supernatant and inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Supelcosil LC-18-DB
Column: Supelcosil LC-18-DB
Mobile phase: MeOH:10 mM pH 4.4 citrate buffer 10:90 containing 10 μM disodium EDTA and 0.5 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 10
Detector: E, EICOM Co. ECD-100, +0.7 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4
Internal standard: isoproterenol (45)

KEY WORDS
plasma; rat; brain; pharmacokinetics

REFERENCE

Sato,S.; Koitabashi,T.; Koshiro,A. Pharmacokinetic and pharmacodynamic studies of L-dopa in rats. I. Pharmacokinetic analysis of L-dopa in rat plasma and striatum, *Biol.Pharm.Bull.*, **1994**, *17*, 1616–1621.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 150 μL Toyopak IC-SP S (sulfopropyl resin, H^+ form) SPE cartridge (Tosoh) with 10 mL water. Plasma. 700 μL Plasma + 50 μL 700 nM 3,4-dihydroxybenzylamine + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min. Add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Urine. Acidify urine collected over 24 h with 10 mL 6 M HCl. 500 μL Urine + 25 μL 10 μM 3,4-dihydroxybenzylamine + 25 μL 40 μM ferulic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)

Mobile phase: Gradient. A was buffer. B was MeCN:MeOH:buffer 8:12:80, pH 3.1. A:B 100:0 for 4 min, to 60:40 over 8 min, to 0:100 over 2 min, maintain at 0:100 for 16 min, return to initial conditions (step gradient), re-equilibrate for 20 min. Buffer was 60 mM pH 3.1 citric acid containing 32 mM Na_2HPO_4, 1.7 mM sodium hexanesulfonate, and 0.1 mM disodium EDTA (J. Chromatogr. 1989, 467, 237).

Flow rate: 1

Injection volume: 50

Detector: F ex 345 em 480 following post-column reaction. The column effluent passed through a Hitachi 655A electrochemical detector with carbon cloth electrodes; working electrode at +0.68 V versus reference electrode (200 mM equimolar mixture of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) containing 200 mM potassium nitrate and 200 mM KOH). The effluent from the electrochemical detector mixed with 20 mM meso-1,2-diphenylethylenediamine in 50 mM HCl pumped at 0.4 mL/min and with 1 M glycine containing 490 mM KOH and 3 mM potassium hexacyanoferrate(III) pumped at 0.4 mL/min. This mixture flowed through a 10 m × 0.47 mm ID coil at 80° to the detector (J. Chromatogr. 1989, 467, 237).

CHROMATOGRAM

Retention time: 9.5

Internal standard: 3,4-dihydroxybenzylamine (12.5)

Limit of detection: 12 nM (urine), 10 nM (plasma)

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, metanephrine, 3-methoxytyramine, norepinephrine

KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Nohta,H.; Yamaguchi,E.; Ohkura,Y.; Watanabe,H. Measurement of catecholamines, their precursor and metabolites in human urine and plasma by solid-phase extraction followed by high-performance liquid chromatography with fluorescence derivatization, *J.Chromatogr.*, **1989**, *493*, 15–26.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 30 μL 700 nM isoproterenol + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot

of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 10 μL 15 μM isoproterenol + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)

Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)

Flow rate: 0.8

Injection volume: 100

Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM

Retention time: 23

Internal standard: isoproterenol (60)

Limit of detection: 7-9 nM

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, metanephrine, 3-methoxytyramine, norepinephrine, normetanephrine

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KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE

Matrix: blood, urine

Sample preparation: 2 mL Plasma or 1 mL urine + dihydroxybenzylamine + 20 mg Sigma WA4 alumina + 200 μL 1 M pH 8.6 Tris-EDTA buffer, mix for 10 min, discard plasma. Wash the alumina three times with 3 mL water and dry it. Add 125 μL 500 mM phosphoric acid, after 1 min inject a 100 μL aliquot. (Ann. Clin. Biochem. 1985, 22, 194-203)

HPLC VARIABLES

Column: 250 × 4.5 5 μm Ultratechsphere

Mobile phase: Per liter 75 mmol citric acid, 58.5 mmol NaH_2PO_4, 0.2 mmol disodium EDTA, and 4.4 mmol heptanesulfonic acid, pH adjusted to 3.4, made up to a final volume of 2 L, add 200 mL MeOH

Flow rate: 1

Injection volume: 100

Detector: E, ESA Coulochem conditioning cell +0.35 V, first electrode +0.05 V, second electrode -0.35 V

CHROMATOGRAM
Retention time: 5.37
Internal standard: dihydroxybenzylamine (10.53)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: norepinephrine, metanephrine, epinephrine, 3-methoxytyrosine, normetanephrine, dihydroxyphenylacetic acid, dopamine

KEY WORDS
plasma

REFERENCE
Dutton,J.; Copeland,L.G.; Playfer,J.R.; Roberts,N.B. Measuring L-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with L-dopa and a dopa decarboxylase inhibitor, *Clin.Chem.*, **1993**, *39*, 629–634.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.575

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or contents of capsules, weigh out an amount equivalent to about 100 mg levodopa, add 30 mL 0.1 M HCl, sonicate, make up to 50 mL with 0.1 M HCl, mix, filter (0.45 μm), discard first 5 mL filtrate. 5 mL Filtrate + 10 mL 2 mg/mL methyldopa in 0.1 M HCl, make up to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 3% aqueous acetic acid

Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3
Internal standard: methyldopa (4.5)

KEY WORDS
capsules; tablets

REFERENCE
Ting,S. Liquid chromatographic determination of levodopa and levodopa-carbidopa in solid dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 987–990.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:50 mM ammonium acetate adjusted to pH 4.1 with 0.6 M acetic acid 1:99
Flow rate: 0.9
Detector: E, Coulochem model 5100A, screen electrode +0.3 V, sample electrode +0.6 V and UV 280

CHROMATOGRAM
Retention time: 6.35
Limit of detection: 1000 ng/mL (UV), 20 ng/mL (E)

OTHER SUBSTANCES
Simultaneous: hydroxydopa, carbidopa, methyldopa, methoxytyrosine, methylcarbidopa, impurities

KEY WORDS
stability-indicating; tablets

REFERENCE
Kafil,J.B.; Dhingra,B.S. Stability-indicating method for the determination of levodopa, levodopa-carbidopa and related impurities, *J.Chromatogr.A*, **1994**, *667*, 175–181.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, isoproterenol, methyldopa, norepinephrine, phenylephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE
Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 1.48, 1.70 (enantiomers)

OTHER SUBSTANCES
Simultaneous: baclofen, norephedrine, primaquine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a 70 × 5 SPE column of Sephadex G 10 in a Pasteur pipette, wash with 3 mL 20 mM ammonia and 3 mL 10 mM formic acid, let stand for 10 days. Homogenize up to 150 mg rat brain in 1 mL 100 mM perchloric acid, centrifuge at 4000 g at 4° for 15 min, add 500 μL of the supernatant to the SPE column, wash with 2.5 mL 10 mM formic acid, elute with 1 mL 10 mM formic acid followed by 1.5 mL 5 mM Na_2HPO_4, inject an aliquot of the eluate.

HPLC VARIABLES
Column: Nucleosil 5 C18
Mobile phase: pH 5.5 Buffer prepared from 200 mM Na_2HPO_4 and 100 mM citric acid
Flow rate: 0.8
Injection volume: 200
Detector: E, rotating disc electrode, 500 mV

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.015 nmole/g

OTHER SUBSTANCES
Extracted: dopamine, uric acid

KEY WORDS
rat; brain; SPE

REFERENCE
Westerink,B.H.C.; Mulder,T.B.A. Determination of picomole amounts of dopamine, noradrenaline, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid in nervous tissue after one-step purification on Sephadex G-10, using high-performance liquid chromatography with a novel type of electrochemical detection, *J.Neurochem.*, **1981**, *36*, 1449–1462.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 100 μL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA + 25 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 25 mg alumina + 1 mL 2 M pH 8.6 Tris-HCl buffer in a microfilter tube (Centrex, Schleicher & Schuell), vortex 5 min, allow to stand for 10 min, filter off water, wash with 5 mL water, add 5 mL water, centrifuge at 3000 g, vortex with 400 μL 0.2 M perchloric acid containing 11 mM disodium EDTA and 0.4 M sodium metabisulfite, centrifuge at 9000 g for 5 min, inject 50 μL of filtrate. (Stabilize each 10 mL urine sample immediately with 0.5 mL 0.1 M HCl and 1 mL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA.)

HPLC VARIABLES
Guard column: 40 × 4.6 Bio-Sil ODS-10 (Bio-Rad)
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:water 22.5:77.5 containing 20 mM citric acid, 20 mM Na_2HPO_4, 4 mM sodium octanesulfonate, and 0.05 mM disodium EDTA, pH adjusted to 2.74 ± 0.01 with 2 M citric acid
Column temperature: 40
Injection volume: 50
Detector: E, BAS LC-4B, 0.54 V vs Ag/AgCl, 50 nA full scale

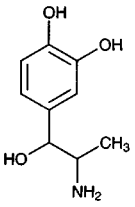
CHROMATOGRAM
Retention time: 6
Internal standard: α-ethyldopa (14)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: carbidopa, dopamine

KEY WORDS
SPE

REFERENCE
Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

Levonordefrin



Molecular formula: $C_9H_{13}NO_3$
Molecular weight: 183.21
CAS Registry No.: 829-74-3, 138-61-4 (racemic HCl)
Merck Index: 6785
Lednicer No.: 1 68

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 12
Limit of detection: 36 ng

OTHER SUBSTANCES

Simultaneous: norepinephrine, epinephrine, isoproterenol, phenylephrine, metaraminol, impurities

KEY WORDS

tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE

Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 12

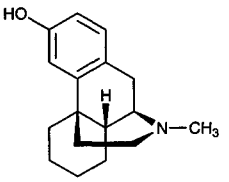
OTHER SUBSTANCES

Simultaneous: epinephrine, isoproterenol, metaraminol, phenylephrine

REFERENCE

Phenomenex Catalog, **1994**, p. 1.077.

Levorphanol



Molecular formula: $C_{17}H_{23}NO$
Molecular weight: 257.38
CAS Registry No.: 77-07-6, 5985-38-6 (tartrate dihydrate), 125-72-4 (tartrate)
Merck Index: 5496
Lednicer No.: 1 293

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 20 ng levallorphan, mix, add 1 mL 1 M pH 9 borate buffer, add 10 mL hexane:ethyl acetate 90:10, shake for 10 min, centrifuge at 2500 rpm for 10 min. Remove 9 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:buffer 30:70 containing 0.1 mM EDTA (Buffer was 10 mM NaCl adjusted to pH 4.8 with 1 M HCl.)
Flow rate: 1
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode +1.00 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6.5
Internal standard: levallorphan (9.5)
Limit of quantitation: 1.25 ng/mL

OTHER SUBSTANCES

Simultaneous: 6-acetylmorphine, diamorphine, oxymorphine, pentazocine
Noninterfering: acetaminophen, 1-α-acetylmethodol, caffeine, codeine, hydrocodone, hydromorphine, meperidine, morphine, oxycodone, propoxyphene

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Lucek,R.; Dixon,R. Quantitation of levorphanol in plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *341*, 239–243.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2.58 μg/mL laudanosine in MeCN + 500 μL saturated sodium carbonate solution, vortex for 10 s, add 5 mL chloroform, vortex for 10 s, mix on a rocking mixer for 40 min, centrifuge at 2000 g for 25 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL mobile phase, inject a 300 μL aliquot. (Hydrolyze conjugates by heating 1 mL plasma with 1 mL 3000 U/mL β-glucuronidase (Helix pomatia type H-1 (Sigma)) in 100 mM pH 5.0 sodium citrate at 37° for 2 h, proceed as above.)

HPLC VARIABLES

Column: 150 × 4.6 Spherisorb 5-CN
Mobile phase: MeCN:water:triethylamine 10:89:1 adjusted to pH 6 with orthophosphoric acid
Flow rate: 1
Injection volume: 300
Detector: F ex 280 em 315

CHROMATOGRAM
Retention time: 6.615
Internal standard: laudanosine (8.603)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: guaifenesin

KEY WORDS
plasma

REFERENCE
Stavchansky,S.; Demirbas,S.; Reyderman,L.; Chai,C.-K. Simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 919–925.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Dilute urine 3:1 or more with water. Vortex 1 mL CSF, plasma, or diluted urine with 100 μL 100 ng/mL IS, add 500 μL saturated sodium carbonate, mix, add 5 mL hexane containing 0.1% n-octylamine. Vortex for 60 s, centrifuge at 2000 g for 10 min. Re-extract aqueous phase with 5 mL hexane containing 0.1% n-octylamine, evaporate the combined hexane extracts to dryness under a stream of nitrogen in a 50° water bath. Reconstitute residue with 150 μL 100 mM HCl, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm CN
Column: 220 × 4.6 5 μm Brownlee Spheri-5CN (Applied Biosystems, USA)
Mobile phase: MeCN:n-octylamine:water 19:0.05:80.95 adjusted to pH 2.8 with phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 5.3
Internal standard: levallorphan (7.4)
Limit of detection: 1 ng/mL
Limit of quantitation: 1 ng/mL (CSF, plasma), 5 ng/mL (urine)

OTHER SUBSTANCES
Extracted: dextromethorphan

KEY WORDS
plasma

REFERENCE
Kimiskidis,V.K.; Kazis,A.D.; Niopas,I. Simultaneous determination of dextromethorphan and dextrorphan in human plasma, urine and cerebrospinal fluid by HPLC with fluorescence detection, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 1267–1275.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL 1 μg/mL pholcodine in water + 500 μL saturated sodium carbonate, mix, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 5 min, inject a 10-50 μL aliquot of the aqueous layer. Urine. 500 μL Urine + 50 μL 50 μg/mL pholcodine in water + 500 μL saturated sodium carbonate, mix, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix on a rotary mixer for 10

min, centrifuge at 2000 g for 5 min, inject a 10-50 μL aliquot of the aqueous layer. (If desired, hydrolyse 500 μL plasma or urine with 500 μL 8000 U/mL β-glucuronidase (Helix pomatia, type H-1, Sigma) in 200 mM pH 5 acetate buffer at 37° for 16 h, proceed as above.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:water:triethylamine 17:82.94:0.06, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 1
Injection volume: 10-50
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 5.1
Internal standard: pholcodine (6.3)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan
Noninterfering: acetaminophen, clofibrate, codeine, cyclophosphamide, diclofenac, digoxin, doxepin, doxorubicin, estrogens, flucloxacillin, folic acid, furosemide, metformin, metoclopramide, miconazole, minoxidil, morphine, nifedipine, nitroglycerin, norcodeine, norethisterone, oxazepam, oxethazaine, prednisolone, pseudoephedrine, quinine, spironolactone, temazepam, tolbutamide, warfarin

KEY WORDS
plasma

REFERENCE
Chen,Z.R.; Somogyi,A.A.; Bochner,F. Simultaneous determination of dextromethorphan and three metabolites in plasma and urine using high-performance liquid chromatography with application to their disposition in man, *Ther.Drug Monit.*, **1990**, *12*, 97–104.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 600 μL Microsomal incubation + 600 μL saturated sodium carbonate, place on ice, add 150 μL 12.5 μg/mL betaxolol, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 300 μL 0.5% orthophosphoric acid, extract, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 5 mm i.d. 4 μm Nova-Pak phenyl radial-Pak
Mobile phase: MeCN:MeOH:0.05% orthophosphoric acid 24:10:66
Flow rate: 1.6
Detector: F ex 261 em 306

CHROMATOGRAM
Retention time: 5.5
Internal standard: betaxolol
Limit of detection: 60 nM

OTHER SUBSTANCES
Extracted: dextromethorphan

KEY WORDS
rat; liver

REFERENCE
Laslett,T.J.; Alvarez,F.; Nation,R.L.; Evans,A.M.; Scott,S.D.; Stupans,I. Effect of cyclophosphamide administration on the activity and relative content of hepatic P4502D1 in rat, *Xenobiotica*, **1995**, *25*, 1031–1039.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.55

OTHER SUBSTANCES
Simultaneous: buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 2.755

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8

Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.26

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine
Sample preparation: 1 mL Urine + 1 mL 100 mM pH 5 acetate buffer + 20 μL β-glucuronidase/β-arylsulfatase (Helix pomatia, Boehringer Mannheim) + 50 μL 600 mM sodium azide in water, heat at 37° for 12 h. Inject a 100 μL aliquot onto column A and elute to waste at 0.5 mL/min, after 3 min elute contents of column A onto column B at 1.4 mL/min, monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 × 4 10 μm LiChrosorb CN; B 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 50:50, adjusted to pH 4 with phosphoric acid
Flow rate: A 0.5; B 1.4
Injection volume: 100
Detector: UV 200 or F ex 280 em 310

CHROMATOGRAM
Retention time: 9.5
Limit of detection: <50 ng/mL (UV)

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan

KEY WORDS
column-switching

REFERENCE
Motassim,N.; Decolin,D.; Le Dinh,T.; Nicolas,A.; Siest,G. Direct determination of dextromethorphan and its three metabolites in urine by high-performance liquid chromatography using a precolumn switching system for sample clean-up, *J.Chromatogr.*, **1987**, *422*, 340–345.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut silica modified with carboxylic acid ion-exchange groups SPE cartridge with 1 mL MeCN:100 mM HCl 40:60 and 1 mL water. Adjust 1 mL urine to pH 5.0-5.5, add β-glucuronidase/arylsulfatase (Helix pomatia (Boehringer Mannheim), heat at 37° for 18 h, add 1 mL to the SPE cartridge, wash with 1 mL water, wash with 500 μL 100 mM HCl, elute with 1 mL MeCN:100 mM HCl 40:60, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 mm long 5 μm Zorbax phenyl
Mobile phase: MeCN:100 mM KH_2PO_4 45:55, adjusted to pH 4
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 310 or UV 280

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 20 ng/mL (F)

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan

KEY WORDS
SPE

REFERENCE
Jacqz-Aigrain,E.; Menard,Y.; Popon,M.; Mathieu,H. Dextromethorphan phenotypes determined by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1989**, *495*, 361–363.

SAMPLE
Matrix: urine
Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 6 mL MeOH, 6 mL water, and 4 mL 100 mM pH 9.2 sodium carbonate buffer. 750 μL Urine + 750 μL 100 mM pH 5.0 sodium acetate buffer containing 20 μL β-glucuronidase-arylsulfatase (Helix pomatia, 100000 Fisherman units/mL, Boehringer Mannheim) + 50 μL 600 mM sodium azide, heat at 37° for 18 h. 250 μL Hydrolysed urine + 100 μL 10 μg/mL levallorphan tartrate in water + 2 mL 100 mM pH 9.2 sodium carbonate, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN, elute with 3 mL MeOH:MeCN:2% phosphoric acid 50:30:20. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot. (For low concentrations of dextromethorphan: 500 μL Hydrolysed urine + 100 μL 1 μg/mL levallorphan tartrate in water + 2 mL 100 mM pH 9.2 sodium carbonate, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN, elute with 3 mL MeOH:MeCN:2% phosphoric acid 50:30:20. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 120 μL aliquot.)

HPLC VARIABLES
Guard column: 15 × 3.2 RP-2 (Brownlee)
Column: 250 × 4.6 5 μm Zorbax phenyl
Mobile phase: MeCN:MeOH:10 mM pH 2.5 phosphate buffer containing 2.5 mM 1-octanesulfonic acid 27:13:60
Column temperature: 30
Flow rate: 1
Injection volume: 20-120
Detector: F ex 270 em 312

CHROMATOGRAM
Retention time: 7.0
Internal standard: levallorphan tartrate (9.5)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan

KEY WORDS
SPE

REFERENCE
Wenk,M.; Todesco,L.; Keller,B.; Follath,F. Determination of dextromethorphan and dextrorphan in urine by high-performance liquid chromatography after solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 341–344.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 1.25 μg levallorphan + 250 μL 140 mM pH 5 sodium acetate buffer, mix, add 25 μL β-glucuronidase (glucurase, from bovine liver, 5000 U/mL, Sigma), heat at 37° overnight, add 1.5 mL pH 11.3 glycine buffer, add 6 mL hexane:butanol 90:10, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: Nucleosil 7 C6H5
Mobile phase: MeCN:MeOH:buffer 20:10:70 (Buffer was 10 mM phosphate buffer containing 2.5 mM sodium 1-octanesulfonate, adjusted to pH 2.5 with concentrated phosphoric acid.)
Flow rate: 1.3
Injection volume: 10
Detector: F ex 270 em 312

CHROMATOGRAM
Retention time: 6
Internal standard: levallorphan (9)

OTHER SUBSTANCES
Extracted: dextromethorphan

REFERENCE
Caslavska,J.; Hufschmid,E.; Theurillat,R.; Desiderio,C.; Wolfisberg,H.; Thormann,W. Screening for hydroxylation and acetylation polymorphisms in man via simultaneous analysis of urinary metabolites of mephenytoin, dextromethorphan and caffeine by capillary electrophoretic procedures, *J.Chromatogr.B*, **1994**, *656*, 219–231.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 5000 U β-glucuronidase in 1 M pH 5.0 sodium acetate buffer, heat at 37° for 18 h, add 500 μL saturated sodium carbonate, add 10 mL hexane:

triethylamine 99.9:0.1. Remove the organic layer, dry, reconstitute the residue in 250 μL mobile phase, inject a 5-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Selectosil (Phenomenex)
Mobile phase: MeCN:10 mM pH 3.0 potassium phosphate buffer 30:70
Flow rate: 1
Injection volume: 5-50
Detector: F ex 280 em 305

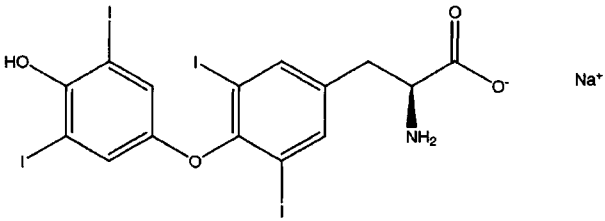
CHROMATOGRAM
Limit of detection: 2 μM

OTHER SUBSTANCES
Extracted: dextromethorphan

REFERENCE
Marinac,J.S.; Foxworth,J.W.; Willsie,S.K. Dextromethorphan polymorphic hepatic oxidation (CYP2D6) in healthy black american adult subjects, *Ther.Drug Monit.*, **1995**, *17*, 120–124.

Levothyroxine sodium

Molecular formula: $C_{15}H_{10}I_4NNaO_4$
Molecular weight: 798.86
CAS Registry No.: 55-03-8, 25416-65-3 (hydrate), 51-48-9 (free acid)
Merck Index: 5497
Lednicer No.: 1 97



SAMPLE
Matrix: blood
Sample preparation: Equilibrate a Sep-Pak silica cartridge with 5 mL ethyl acetate. 1 mL Serum + 3 mL 5% trichloroacetic acid + 4 mL ethyl acetate, vortex vigorously, centrifuge at 1500 g for 5 min. Remove organic layer and repeat extraction twice with 3 mL portions of ethyl acetate. Combine extracts, evaporate to about 1.5 mL, add to Sep-Pak cartridge. Wash with 8 mL ethyl acetate, elute with 4 mL MeOH:ammonium hydroxide (90:10). Evaporate the eluent to dryness under nitrogen, reconstitute in 100 μL MeOH, inject.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere I.P.
Mobile phase: MeCN:buffer 35:65 (Buffer was 13.6 g sodium acetate, 1.0 g cupric sulfate pentahydrate, 0.92 g L-proline, and 0.34 g silver nitrate.)
Flow rate: 1.5
Injection volume: 100
Detector: E, Bioanalytical Systems Inc. TL-5 Kel-F glassy carbon thin-layer cell, LC-4 electronic controller, +0.78 V, 2-5 nA/V

CHROMATOGRAM
Retention time: 8
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: dextrothyroxine, triiodothyronine

KEY WORDS
serum; SPE

REFERENCE
Hay,I.D.; Annesley,T.M.; Jiang,N.S.; Gorman,C.A. Simultaneous determination of D- and L-thyroxine in human serum by liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1981**, *226*, 383–390.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out powder equivalent to about 65 mg thyroid, add 5 mL enzyme solution, mix well, incubate at 37° for 28 h, agitate after 4-8 h and after 20-24 h, add 2 mL deactivating solution, mix well, centrifuge at 2000 rpm for 5-10 min, if necessary filter (0.45 μm). (The enzyme solution was about 150 protease units/mL of bacterial protease from Streptomyces griseus in 110 mM NaCL + 40 mM Tris buffer + 50 mM methimazole (pH adjusted to 8.4 ± 0.05 with 6 M HCl) reducing buffer. Deactivating solution was 1:100 phosphoric acid: MeCN.)

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:0.5% phosphoric acid in water 28:72
Column temperature: 34
Flow rate: 1.5
Injection volume: 200
Detector: UV 225

CHROMATOGRAM
Retention time: 22

OTHER SUBSTANCES
Simultaneous: liothyronine, L-3,3',5'-triiodothyronine

KEY WORDS
tablets

REFERENCE
Richheimer,S.L.; Jensen,C.B. Determination of liothyronine and levothyroxine in thyroid preparations by liquid chromatography, *J.Pharm.Sci.*, **1986**, *75*, 215–217.

SAMPLE
Matrix: formulations
Sample preparation: Grind a tablet, add 50 μg 3,3',5'-triiodothyronine, add 20 mL solvent A, stir for 10 min, add 40 mL solvent B, stir for 30 min, filter. Remove the upper layer and wash it six times with 15 mL portions of water saturated with butanol, evaporate under vacuum at 40-42°, reconstitute in 2.5 mL 3% ammonium hydroxide in MeOH, inject an aliquot (Anal.Lett. 1979, 12, 1201). (Prepare the solvents by mixing 1.8 L 1-butanol, 1.35 L water and 450 mL concentrated HCl, shake vigorously for 20 min, allow to separate. The lower layer was solvent A and the upper layer was solvent B.)

HPLC VARIABLES
Guard column: 25 × 2.5 Co:Pel ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:100 mM pH 5.0 ammonium acetate 50:50
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 19.5
Internal standard: 3,3',5'-triiodothyronine

OTHER SUBSTANCES
Simultaneous: liothyronine

KEY WORDS
protect from light; tablets

REFERENCE
Rapaka,R.S.; Knight,P.W.; Prasad,V.K. Reversed-phase high-performance liquid chromatographic analysis of liothyronine sodium and levothyroxine sodium in tablet formulations: preliminary studies on dissolution and content uniformity, *J.Pharm.Sci.*, **1981**, *70*, 131–134.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to about 200 μg sodium levothyroxine, add 10 mL mobile phase, sonicate for 5 min, centrifuge. Filter (0.45 μm, 25 mm Acrodisc CR, Gelman) the supernatant, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 40 μm RP 201SC pellicular (Vydac)
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:buffer 60:40 (Buffer was pH 3.0 containing 5 mM 1-octanesulfonic acid and 5 mM tetramethylammonium chloride.)
Flow rate: 2
Injection volume: 200
Detector: UV 230

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: liothyronine, 3,5-diiodo-L-thyronine

KEY WORDS
tablets; stability-indicating

REFERENCE
Richheimer,S.L.; Amer,T.M. Stability-indicating assay, dissolution, and content uniformity of sodium levothyroxine in tablets, *J.Pharm.Sci.*, **1983**, *72*, 1349–1351.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets containing about 1 mg levothyroxine, add 4.5 mL 0.5 mg/mL hydroxyprogesterone caproate in MeOH, add 20.5 mL 10 mM NaOH in MeOH:water 75:25, shake intermittently for 5 min, filter, discard first 5 mL filtrate, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak CN
Mobile phase: MeCN:0.1% phosphoric acid in water 35:65
Flow rate: 3
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 5
Internal standard: hydroxyprogesterone caproate (8)

KEY WORDS
tablets

REFERENCE
Das Gupta,V.; Odom,C.; Bethea,C.; Plattenburg,J. Effect of excipients on the stability of levothyroxine sodium tablets, *J.Clin.Pharm.Ther.*, **1990**, *15*, 331–336.

SAMPLE
Matrix: formulations

Sample preparation: Condition a 13 mm Empore C18 SPE disk (Baker) with 2.5 mL MeOH and 2.5 mL water at 1.5 mL/min. Dissolve tablet in dissolution medium (?). Pass 40 mL through the SPE disk, wash with 2.5 mL water, dry, add 1 mL MeOH and let it soak in for 3 min, elute at 0.5 mL/min, dilute the eluate to 2 mL with 50 mM pH 2.5 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher cyano
Mobile phase: MeCN:water:phosphoric acid 45:55:0.05
Column temperature: 25
Flow rate: 1.6
Injection volume: 20
Detector: UV 225

KEY WORDS
tablets; SPE; comparison with capillary electrophoresis

REFERENCE
Carducci,C.N.; Lucangioli,S.E.; Rodríguez,V.G.; Fernández Otero,G.C. Application of extraction disks in dissolution tests of clenbuterol and levothyroxine tablets by capillary electrophoresis, *J.Chromatogr.A*, **1996**, *730*, 313–319.

SAMPLE
Matrix: solutions
Sample preparation: Take up 1.5 mg levothyroxine in 200 μL 100 mM sodium bicarbonate and 400 μL reagent, stir in an ice bath for 30 min, evaporate to dryness below 30°, add 100 μL trifluoroacetic acid to the dry residue, let stand for 30 min at room temperature, add 2 mL 1 M sodium bicarbonate, centrifuge. Remove the precipitate and dissolve it in 600 μL MeOH:20 mM NaOH 50:50, inject a 15 μL aliquot. Reagent was 7 mg/mL BOC-L-Leu-SU (tert-butyloxy-L-leucine-N-hydroxysuccinimide ester) in MeOH, prepared immediately before use.)

HPLC VARIABLES
Column: 150 × 3.2 7 μm LiChrosorb RP-18
Mobile phase: MeOH:water 60:40 containing 0.05% methanesulfonic acid
Flow rate: 1
Injection volume: 15
Detector: UV 230

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.05% of the D form

OTHER SUBSTANCES
Simultaneous: dextrothyroxine, impurities

KEY WORDS
derivatization; chiral

REFERENCE
Lankmayr,E.P.; Budna,K.W.; Nachtmann,F. Separation of enantiomeric iodinated thyronines by liquid chromatography of diastereomers, *J.Chromatogr.*, **1980**, *198*, 471–479.

SAMPLE
Matrix: solutions
Sample preparation: 20 μL Solution + 40 μL 50 mM pH 8.5 borate buffer + 40 μL 4 mM dabsyl chloride in MeCN, mix, heat at 70° for 15 min, add 100 μL 25 mM pH 6.5 sodium acetate buffer, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 30 μm Chromspher C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)

Mobile phase: Gradient. A was MeOH:25 mM pH 6.5 sodium acetate buffer 70:30. B was MeOH:25 mM pH 6.5 sodium acetate buffer 90:10. A:B from 100:0 to 0:100 over 15 min, maintain at 0:100 for 5 min.
Column temperature: 35
Flow rate: 0.7
Detector: UV 436

CHROMATOGRAM
Retention time: 14.5
Limit of detection: 0.39 pmole

OTHER SUBSTANCES
Simultaneous: 3,5-diiodothyronine, 3,5-diiodotyrosine, liothyronine, 3-monoiodotyrosine, thyronine, 3,3',5-triiodothyronine, tyrosine

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KEY WORDS
derivatization; comparison with other derivatization procedures

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REFERENCE
Doorn,L.; Jansen,E.H.; Van Leeuwen,F.X. Comparison of high-performance liquid chromatographic detection methods for thyronine and tyrosine residues in toxicological studies of the thyroid, *J.Chromatogr.*, **1991**, *553*, 135–142.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL Solution + 100 μL 500 mM pH 7.7 borate buffer + 100 μL 2.5 mM 9-fluorenylmethyl chloroformate in dry acetone, mix, let stand at room temperature for 45 s, add 200 μL 12 mM 1-adamantamine in MeCN, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 30 μm Chromspher C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: Gradient. A was MeOH:50 mM pH 4.2 sodium acetate buffer 40:60. B was MeCN:MeOH:50 mM pH 4.2 sodium acetate buffer 20:60:20. A:B from 100:0 to 0:100 over 40 min.
Column temperature: 35
Flow rate: 0.7
Detector: UV 260

CHROMATOGRAM
Retention time: 33
Limit of detection: 1.6 pmole

OTHER SUBSTANCES
Simultaneous: 3,5-diiodothyronine, 3,5-diiodotyrosine, liothyronine, 3-monoiodotyrosine, thyronine, 3,3',5-triiodothyronine

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KEY WORDS
derivatization; comparison with other derivatization procedures

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REFERENCE
Doorn,L.; Jansen,E.H.; Van Leeuwen,F.X. Comparison of high-performance liquid chromatographic detection methods for thyronine and tyrosine residues in toxicological studies of the thyroid, *J.Chromatogr.*, **1991**, *553*, 135–142.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Phenomenex cyano-bonded silica
Mobile phase: MeCN:water:phosphoric acid 400:600:1

Flow rate: 1.5
Detector: UV 225

CHROMATOGRAM
Retention time: 8.6

OTHER SUBSTANCES
Simultaneous: degradation products, liothyronine

REFERENCE
Won,C.M. Kinetics of degradation of levothyroxine in aqueous solution and in solid state, *Pharm.Res.*, **1992**, *9*, 131–137.

SAMPLE
Matrix: tissue
Sample preparation: 100 µL Thyroid tissue + 200 µL MeCN, mix, centrifuge. Remove a 100 µL aliquot of the supernatant and add it to 100 µL 4 nM dabsyl chloride in MeCN, heat at 70° for 10 min, add 400 µL MeOH:50 mM pH 7.0 phosphate buffer 50:50, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Hypersil ODS
Mobile phase: Gradient. A was MeOH:25 mM pH 6.5 sodium acetate 56:44. B was MeOH. A:B from 80:20 to 35:65 over 15 min, maintain at 35:65 for 3 min, to 0:100 over 1 min, maintain at 0:100 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 436

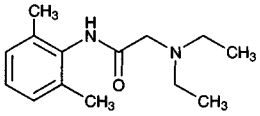
CHROMATOGRAM
Retention time: 17.5

OTHER SUBSTANCES
Extracted: diiodothyronine (T2), liothyronine (T3)

KEY WORDS
derivatization; thyroid

REFERENCE
Jansen,E.H.J.M.; van den Berg,R.H.; Both-Miedema,R.; Doorn,L. Advantages and limitations of pre-column derivatization of amino acids with dabsyl chloride, *J.Chromatogr.*, **1991**, *553*, 123–133.

Lidocaine



Molecular formula: $C_{14}H_{22}N_2O$
Molecular weight: 234.34
CAS Registry No.: 137-58-6, 6108-05-0 (HCl monohydrate), 73-78-9 (HCl)
Merck Index: 5505
Lednicer No.: 1 16

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 µL 2 µg/mL IS in MeOH, add 2 mL water and 2 mL MeCN, vortex gently, set aside for 3 min, centrifuge at 2200 g for 20 min. Separate the clear supernatant, add 500 µL 200 mM NaOH and extract with 6 mL n-hexane by vortexing for 2 min. Centrifuge at 2200 g for 15 min. Evaporate 5 mL of the organic phase to dryness under reduced pressure. Reconstitute the residue in 120 µL mobile phase. Inject a 100 µL aliquot.